Abstract

The present invention relates to methods for cryopreserving plant cells and to methods for recovering viable plant cells from long or short term cryopreservation. Plant cells to be cryopreserved can be grown in culture and pretreated with a solution containing an cryoprotective agent and, optionally, a stabilizer. Stabilizers are preferably membrane stabilizers such as ethylene inhibitors, oxygen radical scavengers and divalent cations. Cells can also be stabilized by subjecting the culture to a heat shock. Pretreated cells are acclimated to a reduced temperature and loaded with a cryoprotective agent such as DMSO, propylene glycol or polyethylene glycol. Loaded cells are incubated with a vitrification solution which, for example, comprises a solution with a high concentration of the cryoprotective agent. Vitrified cells retain less than about 20% water content and can be frozen at cryopreservation temperatures for long periods of time without significantly altering the genotypic or phenotypic character of the cells. Plant cells may also be cryopreserved by lyophilizing cells prior to exposure to a vitrification solution. The combination of lyophilization and vitrification removes about 80% to about 95% of the plant cell's water. Cells can be successfully cryopreserved for long periods of time and viably recovered. The invention also relates to methods for the recovery of viable plant cells from cryopreservation. Cells are thawed to about room temperature and incubated in medium containing a cryoprotective agent and a stabilizer. The cryoprotective agent is removed and the cells successfully incubated and recovered in liquid or semi-solid growth medium. The invention also relates to the cryopreserved cells and to viable plant cells which have been recovered from long or short term cryopreservation.